(i) Results of tests and assays on the batch for potency, sterility, pyrogens, loss on drying, pH, residue on ignition, identity, crystallinity, and kanamycin B content.

(ii) Samples required:

(*a*) For all tests except sterility: 10 packages, each containing approximately 500 milligrams.

(*b*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient sterile distilled water to give a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 10 micrograms of kanamycin per milliliter (estimated).

(2) *Sterility*. Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) [Reserved]

(4) *Pyrogens*. Proceed as directed in § 436.32(b) of this chapter, using a solution containing 10 milligrams of kanamycin per milliliter.

(5) *Loss on drying*. Proceed as directed in § 436.200(b) of this chapter.

(6) *pH*. Proceed as directed in § 436.202 of this chapter, using a solution containing 10 milligrams per milliliter.

(7) *Residue on ignition*. Proceed as directed in § 436.207(a) of this chapter.

(8) *Identity*. Dissolve about 10 milligrams of kanamycin sulfate in 1 milliliter of water and add 1 milliliter of a 1:500 solution of triketohydrindene hydrate in normal butyl alcohol. Then add 0.5 milliliter of pyridine. Heat in a steam bath for 5 minutes and add 10 milliliters of water; a deep-purple color is produced.

(9) *Kanamycin B content*. Proceed as directed in § 444.30(b)(7).

(10) *Crystallinity*. Proceed as directed in § 436.203(a) of this chapter.

[39 FR 19046, May 30, 1974, as amended at 50 FR 19919, May 13, 1985]

§ 444.42 Neomycin sulfate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Neomycin sulfate is the sulfate salt of a kind of neomycin or a mixture of two or more such salts. It is so purified and dried that:

(i) Its potency is not less than 600 micrograms of neomycin per milligram, calculated on an anhydrous basis.

(ii) [Reserved]

(iii) Its loss on drying is not more than 8.0 percent.

(iv) Its pH in an aqueous solution containing 33 milligrams per milliliter is not less than 5.0 and not more than 7.5.

(v) It gives a positive identity test for neomycin.

(2) *Labeling*. It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples*. In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, loss on drying, pH, and identity.

(ii) Samples required: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with solution 3 to the reference concentration of 1.0 microgram of neomycin per milliliter (estimated).

(2) [Reserved]

(3) *Loss on drying*. Proceed as directed in § 436.200(a) of this chapter.

(4) *pH*. Proceed as directed in § 436.202 of this chapter, using a solution containing 33 milligrams of neomycin per milliliter.

(5) *Identity*—(i) *Reagents*. (*a*) Sulfuric acid solution: Mix concentrated sulfuric acid and distilled water in volumetric proportions of 40:60.

(*b*) Xylene.

(*c*) *p*-Bromoaniline: (Prepare and store this reagent in brown, nonactinic glassware.) Place 380 milliliters of thiourea-saturated glacial acetic acid solution in the bottle, add 10 milliliters of

20 percent sodium chloride solution, 5 milliliters of 5 percent oxalic acid solution, and 5 milliliters of 10 percent disodium phosphate solution, and mix well. Add 8 grams of *p*-bromoaniline and mix well. Let this reagent stand overnight before use. Prepare the reagent once weekly.

(ii) *Procedure.* Place about 10 milligrams of the sample into a test tube (19 millimeters × 150 millimeters), dissolve with 1 milliliter of water, and then carefully add 5 milliliters of the sulfuric acid solution. Heat in a boiling water bath for 100 minutes. Cool to room temperature. Add 10 milliliters of xylene to the test tube. Stopper the tube and shake vigorously for about 1 minute. Let the two layers separate and then decant the xylene layer into a second test tube. Add 10 milliliters of the *p*-bromoaniline reagent to the xylene solution, shake, and let stand. The development of a vivid pink-red color is a positive identity test for neomycin.

[40 FR 22252, May 22, 1975, as amended at 50 FR 19919, May 13, 1985]

§ 444.42a Sterile neomycin sulfate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Neomycin sulfate is the sulfate salt of a kind of neomycin or a mixture of two or more such salts. It is so purified and dried that:

(i) It has a potency of not less than 600 micrograms of neomycin per milligram, calculated on an anhydrous basis.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) [Reserved]

(v) Its moisture content is not more than 8.0 percent.

(vi) Its pH in an aqueous solution containing 33 milligrams per milliliter is not less than 5.0 and not more than 7.5.

(vii) It gives a positive identity test for neomycin.

(2) *Labeling.* It is to be labeled in accordance with the requirements of § 432.5(b) of this chapter.

(3) *Request for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, moisture, pH, and identity.

(ii) Samples required;

(*a*) For all tests except sterility: 10 packages, each containing approximately 300 milligrams.

(*b*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Use either of the following methods:

(i) *Plate assay using Staphylococcus epidemmidis (ATCC 12228)*[1] (*a*) *Cylinders (cups).* Use cylinders described in § 440.80a(b)(1)(i) of this chapter.

(*b*) *Culture media.* Using ingredients that conform to the standards prescribed by the U.S.P. or N.F.:

(*1*) Make nutrient agar for carrying the test organism as follows:

Peptone	6.0 gm.
Pancreatic digest of casein	4.0 gm.
Yeast extract	3.0 gm.
Beef extract	1.5 gm.
Dextrose	1.0 gm.
Agar	15.0 gm.
Distilled water q.s	1,000.0 ml.

pH 6.5 to 6.6 after sterilization.

(*2*) Make nutrient agar for the base and seed layers as described in paragraph (b)(1)(i)(*b*)(*1*) of this section, except that its pH after sterilization is 7.8 to 8.0.

In lieu of preparing the media from the individual ingredients specified in paragraph (b)(1)(i)(*b*) of this section, they may be made from a dehydrated mixture that, when reconstituted with distilled water, has the same composition as such media. Minor modification of the individual ingredients specified in paragraph (b)(1)(i)(*b*) of this section are permissible if the resulting media possess growth-promoting properties at least equal to the media described.

(*c*) *Working standard.* Dry a portion of the working standard for 3 hours at 60° C. and a pressure of 5 millimeters or less. Determine the dry weight, and dissolve in sufficient 0.1*M* potassium phosphate buffer, pH 8.0, to give a

[1] Available from: American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852.